Stem Cell Reports
Report

ISSCR

OPEN ACCESS

Intestinal Stem Cell Pool Regulation in *Drosophila*

Yinhua Jin,[1,2,4] Parthive H. Patel,[1,2,4] Alexander Kohlmaier,[1,2] Bojana Pavlovic,[1,2] Chenge Zhang,[1,2] and Bruce A. Edgar[1,2,3,*]

[1]Center for Molecular Biology, University of Heidelberg (ZMBH)
[2]German Cancer Research Center (DKFZ)
69120 Heidelberg, Germany
[3]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA
[4]Co-first author
*Correspondence: bruce.edgar@hci.utah.edu
http://dx.doi.org/10.1016/j.stemcr.2017.04.002

SUMMARY

Intestinal epithelial renewal is mediated by intestinal stem cells (ISCs) that exist in a state of neutral drift, wherein individual ISC lineages are regularly lost and born but ISC numbers remain constant. To test whether an active mechanism maintains stem cell pools in the *Drosophila* midgut, we performed partial ISC depletion. In contrast to the mouse intestine, *Drosophila* ISCs failed to repopulate the gut after partial depletion. Even when the midgut was challenged to regenerate by infection, ISCs retained normal proportions of asymmetric division and ISC pools did not increase. We discovered, however, that the loss of differentiated midgut enterocytes (ECs) slows when ISC division is suppressed and accelerates when ISC division increases. This plasticity in rates of EC turnover appears to facilitate epithelial homeostasis even after stem cell pools are compromised. Our study identifies unique behaviors of *Drosophila* midgut cells that maintain epithelial homeostasis.

INTRODUCTION

Intestinal epithelia are known to require nearly constant self-renewal, supported by small populations of intestinal stem cells (ISCs). Homeostasis in the mammalian intestine is maintained by ISCs that reside in crypts. Two distinct types of stem cells have been reported to maintain ISC pools in the mouse small intestine: the LGR5$^+$ crypt basal columnar cells (CBCs) (Barker, 2014), and the Bmi1$^+$ "+4" cells (Yan et al., 2012), that are positioned just above CBCs. The stem cell population is maintained mainly by asymmetric ISC divisions that generate one new ISC and one cell destined to differentiate. However, it has been reported that LGR5$^+$ crypt cells also divide symmetrically at a high frequency, to generate either two stem cells or two transient amplifying (TA) cells (Barker, 2014; Lopez-Garcia et al., 2010; Snippert et al., 2010). This condition gives rise to "neutral drift" whereby individual ISC lineages are regularly extinguished and replaced, and homeostasis in the stem cell pool size is maintained by an even balance between ISC losses and duplications. Importantly, it was also found that "reserve" +4 stem cells can revert to stem cells following injury, and that this is important in maintaining the stem cell pool (Buczacki et al., 2013; Tian et al., 2011). Moreover, recent work has also shown that even more differentiated cells, including LGR5$^-$ TA cells and Paneth cells, could de-differentiate into LGR5$^+$ cells when they were exposed to WNT3A or following irradiation injury, respectively (Sato et al., 2011; Roth et al., 2012).

As in mammals, epithelial turnover in the *Drosophila* intestine is also mediated by ISCs. Fly ISCs undergo cell division to renew themselves and give rise to transient cells, enteroblasts (EBs), which can differentiate into absorptive enterocytes (ECs) or secretory enteroendocrine (EE) cells. ISCs in the fly midgut express the Notch ligand Delta (Dl), while the major subset of EBs that differentiate into ECs can be identified by their expression of the Notch signal reporter *Suppressor-of-Hairless* [*Su*(*H*)]*GBE-LacZ* (Micchelli and Perrimon, 2006). The transcription factor, *escargot* (*esg*), is required for progenitor identity and marks both ISCs and EBs (Micchelli and Perrimon, 2006). Clonal analysis in the fly midgut shows that 70%–90% of ISC divisions give rise to asymmetric lineages (de Navascues et al., 2012; Micchelli and Perrimon, 2006; O'Brien et al., 2011; Ohlstein and Spradling, 2006, 2007). However, long-term clonal tracing showed that many clones include two or more Dl$^+$ cells, while no Dl$^+$ cells were seen in other multicellular clones (de Navascues et al., 2012). This suggested that ISCs also generate symmetric lineages wherein ISCs either duplicate or fail to self-renew at division. Interestingly, de Navascues et al. (2012) concluded that rates of ISC duplication and loss were equal, such that ISC pools remained constant but lineages were in a state of neutral drift. However, how the fly gut reacts following stem cell depletion has not been intensively investigated, and it is not clear whether active mechanisms exist to maintain stem cell pools following stem cell depletion, such as happens following gut injury or chemotherapy in humans. In addition, whether another cell type could serve as a reserve stem cell pool as in the mammalian intestine has not been investigated. In this study we investigated stem cell pool maintenance in *Drosophila* by quantifying the stem cell number after p53-induced ablation. We found, somewhat surprisingly, that the fly's ISC population is not as actively

maintained as in the mammalian intestine. Following partial ISC ablation, ISCs continued with the normal asymmetric division pattern, and stem cell pools remained reduced over the animal's lifespan. This reduction in ISC pools was, however, compensated for by lower rates of EC loss, allowing maintenance of the organ despite the relative loss in stem cell function.

RESULTS

ISCs Cannot Regenerate after Complete Ablation

We previously found that expressing the cell death effectors *p53*, *ricin A*, or *diphtheria toxin*, in ISCs and their undifferentiated daughters, the EBs, using the *esgGal4 tub-Gal80^{ts}* (*esg^{ts}*) system was effective in removing most of the *esg*$^+$ progenitor cells (Jiang et al., 2009). Surprisingly, another apoptosis effector, *reaper*, was not effective in killing fly ISCs. After 15 days of *p53* induction we noted that virtually all *esg*$^+$ cells had been ablated. Notably, after 15–30 days of continuous *p53* induction, we found that midguts were detectably shrunken with fewer ECs and EEs (Jiang et al., 2009) (Figures S4C and S4D–S4G). To determine whether the midgut could be repopulated with stem cells after complete ISC ablation, we ablated virtually all progenitor cells by expressing *p53* for 15 days and then extinguished *p53* expression for 2 or 4 weeks to allow recovery. No new *esg*$^+$ progenitor cells appeared during this recovery period, implying that a population of *esg*$^-$ cells cannot resupply the midgut with ISCs. In agreement with this, a recent report showed that ISCs failed to regenerate after complete ablation by expressing *prickle-RNAi* in *esg*$^+$ cells (Lu and Li, 2015). As a second test we depleted *esg*$^+$ progenitors by forcing their differentiation into ECs by expressing the intracellular fragment of *Notch* (*Notch^{Intra}*) for 7 days (Figures S1A and S1B). The forced expression of *Notch^{Intra}* in progenitors drives rapid premature differentiation of ISCs into ECs, exhausting the stem cell pool (Micchelli and Perrimon, 2006). Following 7 days of *Notch^{Intra}* expression, we extinguished expression for 2 or 4 weeks and checked for ISC recovery. However, as above, we found that *esg*$^+$ stem cells did not reappear in these midguts (Figure S1C). These data indicate that other *esg*$^-$ cell types, either within or outside the midgut, cannot normally dedifferentiate into *esg*$^+$ progenitor cells. Indeed, we observed midgut atrophy during the recovery period, consistent with permanent progenitor loss and a failure of tissue homeostasis.

ISCs Pools Fail to Recover after Partial Depletion

To study stem cell pool maintenance after partial ISC loss, we expressed *p53* in progenitor cells using the *esg^{ts}* system for 12 hr, 4 days, or 7 days. Numbers of GFP$^+$ cells in posterior midguts were scored. Four- and 7-day inductions of p53 reduced the progenitor cell number by ~50% (Figure 1E and Table S1). To test whether stem cell numbers could recover after their population was diminished by half, we shifted flies to 18°C after 4 or 7 days of *p53* overexpression and monitored the numbers of *esg*$^+$ progenitor cells after 2, 8, 12, 16, or 32 days of recovery. Flies were shifted back to 29°C to induce GFP expression for 12 hr before dissection, a treatment that did not affect ISC numbers (Table S1). These experiments showed that after 8-, 12-, 16-, and 32-day recovery at 18°C, the *esg*$^+$ progenitor cells did not repopulate in either female or male flies (Figures 1A–1E and S1D–S1F). In addition, when *p53* was overexpressed specifically in ISCs using the *esg-Gal4 Su(H)GBE-Gal80 tub-Gal80^{ts}* system (Wang et al., 2014; henceforth referred to as *esg^{ts}*; *Su(H)-Gal80*) or the *Dl-Gal4 tub-Gal80^{ts} UAS-GFP* (*Dl^{ts}*) system, stem cell pools also failed to recover even after 32 days of incubation at 18°C (Figure 1F). These data indicate that ISC pool size is not actively regulated, and suggest that ISCs probably divide asymmetrically even after their population is depleted. These observations also further indicate that *esg*$^-$ cells cannot dedifferentiate into ISCs to maintain the stem cell pool.

ISCs Do Not Compensate for ISC Pool Depletion by Dividing Faster

To determine whether transient p53 expression might affect the behavior of surviving ISCs, we assayed the mitotic index in midguts following partial ISC ablation. Mitotic indices were calculated as the number of PH3$^+$ cells divided by total number of GFP$^+$ cells (ISCs or ISCs + EBs) in the posterior midgut. After ablating ~50% of ISCs, the ISC mitotic index remained significantly similar to that of controls at 2, 16, and 32 days after ISC depletion (Figure 1G). Thus, even though there were fewer ISCs per midgut, these ISCs did not compensate by dividing faster or making more progeny. In addition, we performed partial ISC ablation with p53 and then stimulated ISC divisions with *Pseudomonas entomophila* infection. We found that the remaining ISCs after p53 induction could be induced to divide, like normal control ISCs (Figures 2A–2E). However, as in the previous experiment, the mitotic index of the ISCs that remained after ablation was not increased (Figure 2E). These data indicate that transient p53 expression does not hinder ISC division capability, but also that ISCs do not compensate for ISC pool depletion by dividing more rapidly.

Depleted Stem Cell Pools Do Not Recover during Regeneration

Many investigators have noticed a transient increase in small *esg*$^+$ *Dl*$^+$ cells in the fly midgut during regeneration after damage (Jiang et al., 2009), and this has sometimes been interpreted as an expansion of the ISC pool. Since we

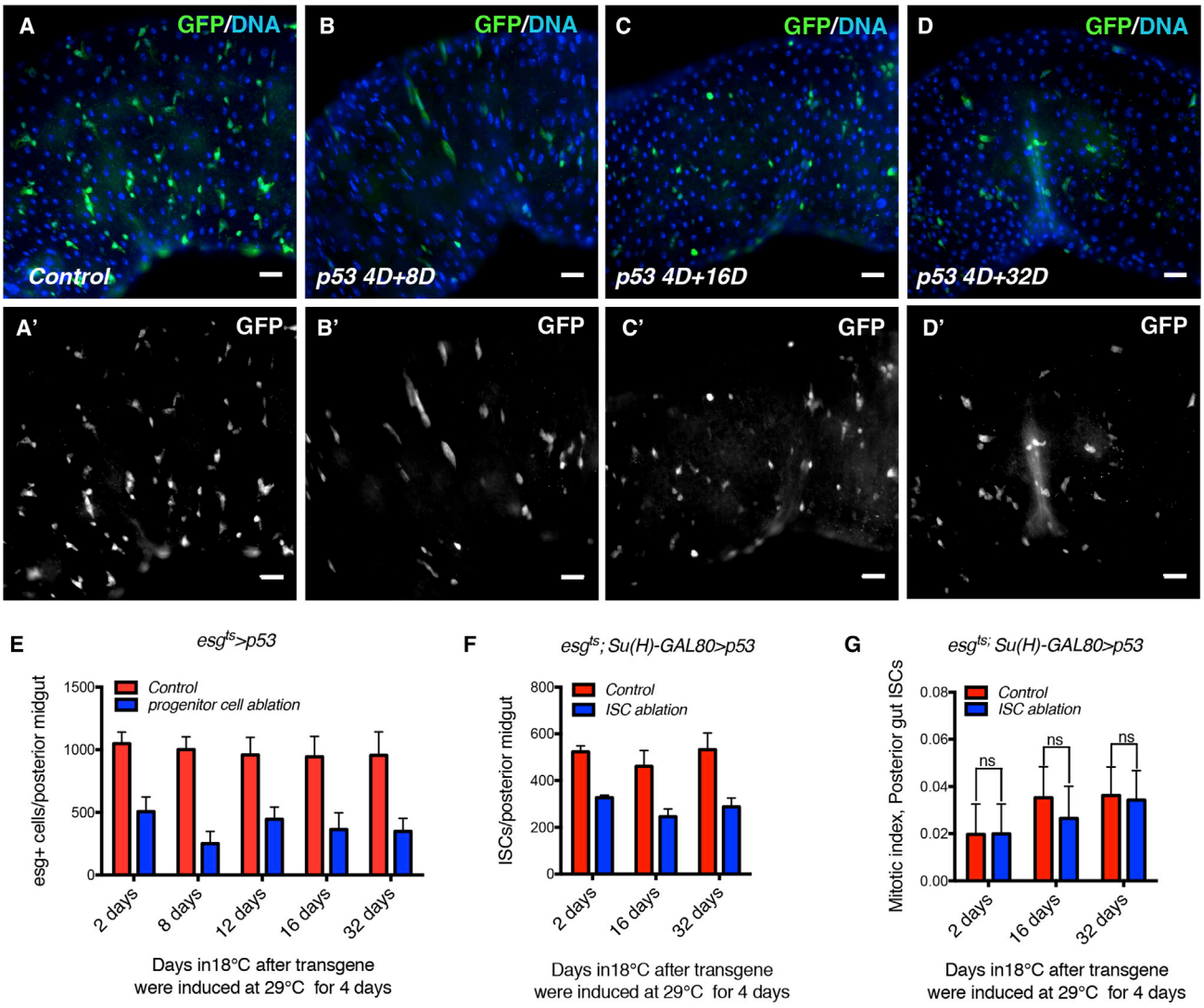


Figure 1. ISCs Failed to Repopulate after the Depletion Induced by *p53* Overexpression

(A–D) ISC and EB maintenance after partial depletion. Transgene expression was induced using the esg^{ts} system at 29°C for 4 days and turned off at 18°C as indicated. GFP expression was induced at 29°C for 12 hr before dissection. (A) Control adult midgut. (B) *p53*-overexpressing midgut after 8-day recovery. (C) *p53*-overexpressing midgut after 16-day recovery. (D) *p53*-overexpressing midgut after 32-day recovery. ISCs and progenitor cells did not repopulate to recover the stem cell pools. Scale bars represent 50 μm.

(E–G) Quantification of GFP^+ progenitors (E), ISCs (F), and mitotic index (G) in posterior midgut during the recovery process both after depletion. n = 10–26 guts; ns (not significant), $p > 0.05$. Error bars in each graph represent SD.

See also Table S1 and Figures S1–S3.

observed this phenomenon in the preceding experiment, we asked whether the increase in esg^+ GFP^+ cells represented stem cell pool recovery during regeneration. *p53* was first induced in progenitor cells for 4 days to deplete the ISCs and then *p53* expression was inhibited for 8 days. Following this, *P. entomophila* was fed to the flies for 2 days to generate an enteric infection, after which kanamycin was fed to the files for another 2 days to kill the *P. entomophila* and clear the infection. The flies were then transferred to standard fly food to recover for 8 or 12 days, and numbers of esg^+ progenitor cells were scored. This experiment detected a transient increase in esg^+ cells and mitoses during the infection, but after clearing the infection the number of esg^+ cells returned to levels seen without infection in both controls and ISC-depleted midguts (Figures 3A, 3B, and S3A–S3D). These data indicate that stem cells pools in ISC-depleted guts do not recover to normal levels, even during stress-dependent

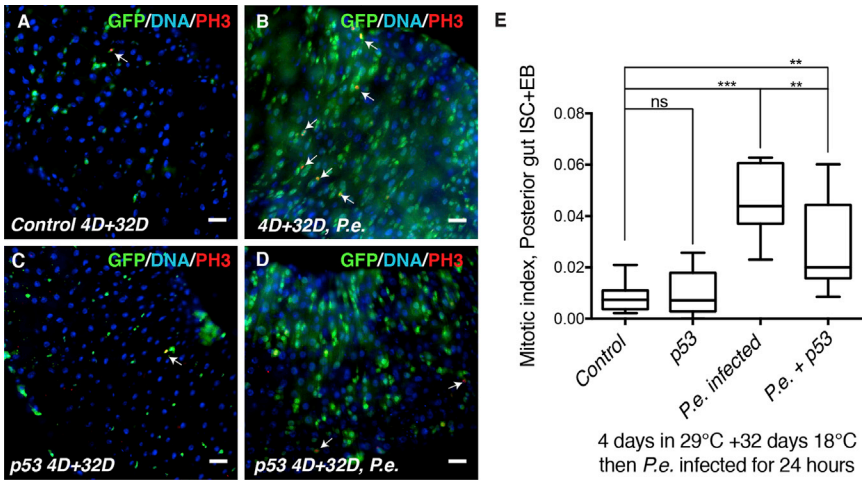


Figure 2. ISCs Still Have the Mitotic Capability after *p53* Overexpression

(A–D) Effect of *P. entomophila* (*P.e.*) infection on ISC proliferation after *p53* overexpression. Transgene expression was induced using the esg^{ts} system at 29°C for 4 days and turned off at 18°C for 32 days. Flies were shifted back to 29°C for *P. entomophila* infection for 24 hr. Samples were stained with anti-GFP (green), anti-PH3 (red, arrow), and DAPI (blue) to mark DNA. Scale bars, 50 μm. (A) Control adult midgut. (B) Control midgut after 24 hr *P. entomophila* infection. (C) *p53*-overexpressing midgut. (D) *p53*-overexpressing midgut after 24 hr *P. entomophila* infection. (E) Quantification of mitotic index of posterior midgut ISCs + EBs following *P. entomophila* infection. Error bars represent SD; n = 10 guts; **p < 0.01, ***p < 0.001. See also Figure S3.

regeneration. Moreover, these results imply that the transient increase in esg^+ cells observed during regeneration does not represent an increase in real stem cell numbers.

ISCs Persist in Asymmetric Division after Infection or EB Depletion

Using the twin-spot MARCM lineage-tracing system (Yu et al., 2009), a previous study determined that insulin signaling could increase rates of symmetric ISC division and thereby expand the stem cell pool (O'Brien et al., 2011). To further explore stem cell behaviors during regeneration following *P. entomophila*-induced epithelial damage, we generated twin-spot MARCM clones 8 hr after *P. entomophila* infection (Figure 3C). By scoring these clones, we found that ~10% of ISC divisions yielded symmetric lineages (*i.e.* ISC duplications) both with or without *P. entomophila* infection (Figure 3D). These data concur with previous observations (de Navascues et al., 2012; O'Brien et al., 2011), and also indicate that most of ISCs continue to undergo asymmetric cell division even during the rapid regeneration that occurs following *P. entomophila*-induced damage. This conclusion is consistent with our previous observation that the number of Dl^+ cells (ISCs) per MARCM clone does not increase following *P. entomophila* infection (Jiang et al., 2009).

Studies in the mouse small intestine reported that Paneth cells, which are an important source of niche factors such as EGF, WNT3A, and Notch ligand, are required for ISC-driven epithelia renewal. Furthermore, Paneth cell ablation blocked stem cell renewal in vivo (Barker, 2014). Based on this example and our observations that EBs express high levels of the ISC mitogen *Spitz*, an EGFR ligand (Dutta et al., 2015), and are physically paired with ISCs, we investigated whether EBs are necessary for ISC renewal or maintenance. We used the EB-specific *Su(H)-Gal4 tubGal80^{ts}* ($Su(H)^{ts}$) system to express *p53* for 30 days. Interestingly, this treatment did not alter EB number, as detected using GFP driven by $Su(H)^{ts}$ (Figure S3E). However, we did observe that the midguts were significantly shrunken, potentially due to a failure of EC replacement. We also observed increased mitoses in $Su(H)^-$ cells, which we assumed to be ISCs (Figure S3F). These data suggest that *p53* probably did ablate $Su(H)^+$ EBs, but that they were rapidly replaced by progeny generated from increased ISC division. If this is the case, a second conclusion would be that EBs are not required for ISC division.

Gut Homeostasis Can Be Maintained by Variations in EC Loss

Previous studies showed that the fly midgut epithelium is constantly renewed, with full replacement taking 1–2 weeks in females (Jiang and Edgar, 2011). It has been assumed that this renewal is necessary because aged and damaged ECs autonomously commit apoptosis and are extruded, and must be replaced. Considering that the EC lifespan is probably limited by intrinsic and extrinsic factors, we posited that the remaining ISCs in ISC-depleted midguts must have to divide more frequently to maintain the epithelium. However, as described above, we found that ISC division rates were not significantly changed following partial ISC ablation (Figures 1G and S3G). Nevertheless, these midguts maintained normal size, cell number, and morphology, suggesting that homeostasis was maintained by another mechanism.

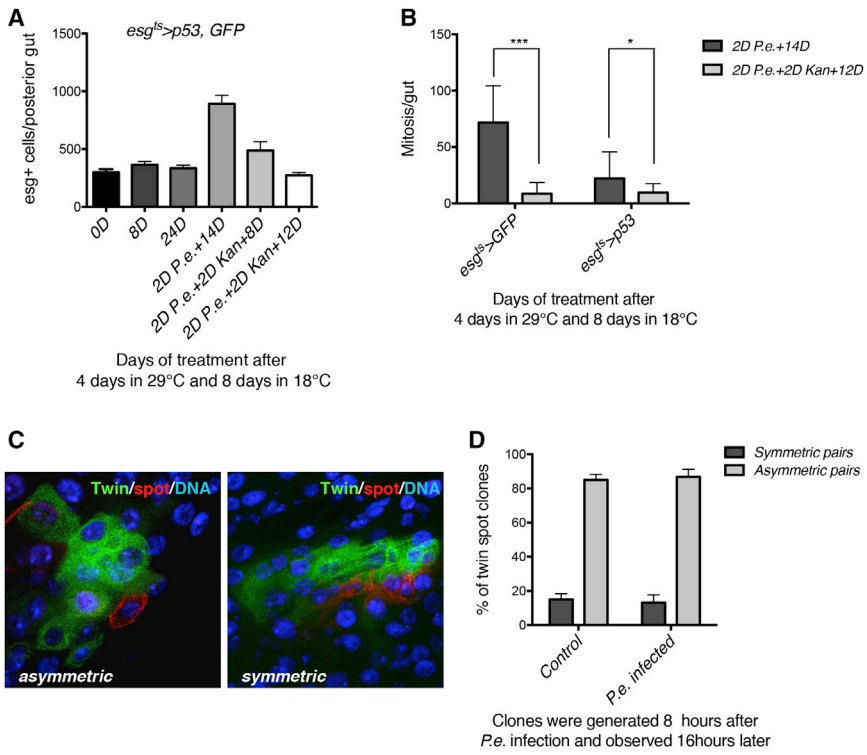


Figure 3. ISCs Divide Asymmetrically Even under Stress Condition

(A) Quantification of GFP$^+$ cells in *p53*-induced midguts (n = 19–26) under different conditions after *p53* was expressed for 4 days and turned off for 8 days.

(B) Quantification of PH3$^+$ cells in *P. entomophila* (*P.e.*)-infected midgut after kanamycin treatment. Induced ISC division upon *P. entomophila* infection was restored after kanamycin treatment. n = 19–25 guts; *p < 0.05, ***p < 0.001.

(C) Two sets of twin-spot MARCM clones are shown. In asymmetric twin spot, the first-born EB differentiated into EC and was labeled with GFP. In symmetric twin spots, both daughter cells generate multicellular clones. Therefore, one was labeled with GFP while the other was labeled with RFP.

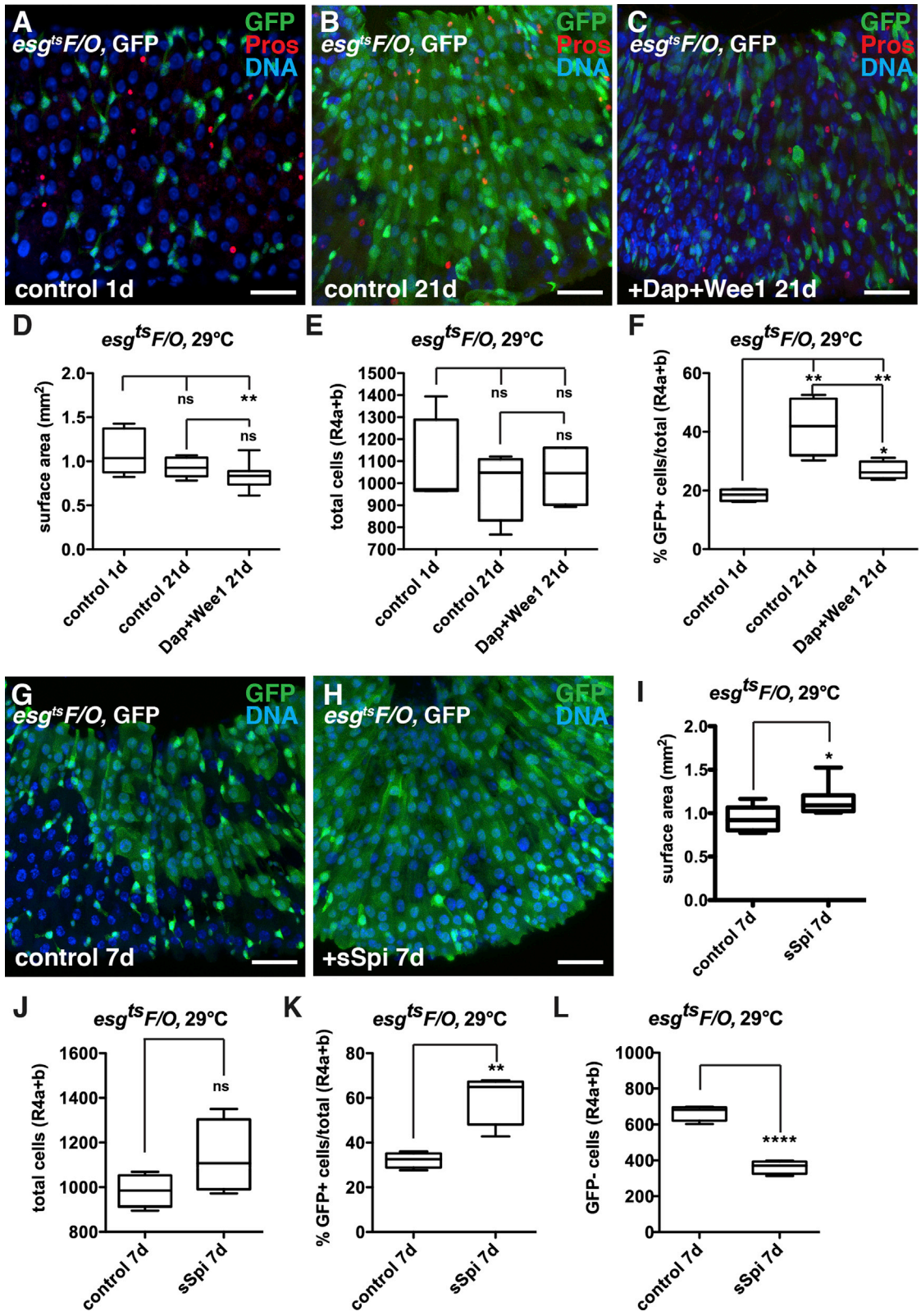
(D) Quantification of the division modes in control (n = 208 clones) and *P. entomophila*-infected (n = 236 clones) midguts (n = 21). Twin spots were induced at the indicated times, and asymmetric and symmetric signatures were scored 16 hr later.

See also Figure S3.

Considering these results, we asked whether changing the rate of ISC divisions might affect the rate of EC loss from the epithelium. To address this possibility we used the *esg^{ts}F/O* system (*esg-GAL4*; *tubGAL80^{ts} Act>Cd2>Gal4 UAS-flp UAS-GFP*) (Jiang et al., 2009) to mark all the ISC progeny produced following expression of genes that would specifically accelerate or retard ISC divisions. Overexpression of the active form of the ISC mitogen Spi (secreted Spi, sSpi) was used to accelerate ISC division, and a combination of *UAS-dap* and *UAS-wee1*, which inhibit Cdk2 and Cdk1, respectively, was used to retard ISC divisions. These treatments should affect only dividing ISCs and differentiating EBs and early ECs but not mature ECs, which are post-mitotic and not responsive to EGFR signaling (Jiang et al., 2011; Xiang et al., 2017). The effects of prolonged ISC cell-cycle acceleration or inhibition on midgut turnover were assessed by measuring overall midgut size (length and surface area) and by counting the number of newly born (GFP$^+$) cells and total cell numbers within defined midgut regions (R4a and R4b). When ISC divisions were inhibited for 21 days by *dap* and *wee1* expression, we found that midgut epithelial turnover was decreased compared with controls (Figures 4A–4C and 4F). Interestingly, the overall midgut size and the total number of R4a + b cells in these midguts remained similar to controls, without detectable gut shrinkage (Figure 4D, 4E, S4A, S4B, and S4D). This result indicates that decelerating ISC division increases EC lifespan and decreases EC loss. Conversely, when ISC divisions were accelerated by enforced sSpi expression, midgut epithelial turnover increased (Figures 4G, 4H, and 4K) and the number of GFP$^-$ cells retained in the epithelium was less than in controls (Figure 4L). Although sSpi expression mildly increased midgut size, the total numbers of R4a + b cells remained similar to what was observed in controls (Figures 4I, 4J, and S4E). This result indicates that accelerating ISC division increases the rate of EC loss. Together these data indicate that the rate of EC loss is regulated by the rate of ISC division, both positively and negatively, and that ECs can persist longer in the gut epithelium if ISC divisions are infrequent. This implies that EC lifespan is determined not only by aging and damage, but also by ISC activity. We infer that, following partial ISC ablation as performed here, epithelial homeostasis is maintained in part by a reduction in the rate of EC loss.

DISCUSSION

To achieve homeostasis in a stem cell pool, stem cell divisions typically give rise to one new stem cell and one cell that is destined to differentiate. This lineage asymmetry can be determined cell-intrinsically, for instance by the asymmetric partitioning of determinants during division,



(legend on next page)

or by localized niche factors. In the latter case, lineage asymmetry may be observed only in populations, rather than by following each and every stem cell division. Studies in mice and flies have documented this sort of population asymmetry in ISC pools and have demonstrated the phenomenon of neutral drift, whereby individual stem cell lineages are born and extinguished at equivalent rates as a result of divisions that either duplicate stem cells or fail at self-renewal. In addition, in mice, dedifferentiation of progenitor cells within the crypt has been observed as a mechanism for restoring lost stem cells. However, the precise response of the gut after stem cell pools are compromised is not well understood in either *Drosophila* or mice. Understanding this response has considerable practical value, since many anti-cancer chemotherapies deplete intestinal and other stem cells, and thereby give rise to debilitating side effects such as gastrointestinal mucositis.

In this study we asked whether the fly intestinal stem cell pool is self-regulatory and capable of regeneration following the ablation of about 50% of the ISCs. Surprisingly, we found that the fly's ISC population did not repopulate itself after ISC depletion. Instead, the remaining ISCs behaved essentially as in normal midguts: they divided at normal rates (Figure 1G) and responded normally to gut epithelial damage with increased division (Figures 2 and 3), but did not duplicate at higher frequencies or regenerate a normal-sized stem cell pool, even after long recovery periods (Figure 1). Nevertheless, midguts with about half the normal ISC number retained their normal size for many weeks, indicating that somehow homeostasis was maintained. In exploring this phenomenon we found that the rate of stem cell division has a strong influence on the rate of loss of differentiated epithelial cells, both when ISC divisions were accelerated or retarded (Figure 4). Hence, we suggest that homeostasis in ISC-depleted guts was made possible by a reduction in the rate of cell loss from the gut epithelium. One explanation for this may be that EC loss rates are substantially determined by ISC division rates, rather than directly by damage from digestive wear and tear and adverse interactions with the gut microbiota, as generally assumed. Our demonstration that EC loss can be accelerated by promoting ISC proliferation is consistent with this view. We speculate that, as between ECs and ISC tumors (Patel et al., 2015), competition between old and newborn ECs for attachment to the basement membrane may underlie these effects on EC lifespan. In support of this, midgut epithelial cell crowding induced by increased ISC proliferation due to stress was shown to be relieved by the loss of excess cells though apoptosis (Loudhaief et al., 2017). The ability to alter the rate of epithelial replacement to match the capabilities of the stem cell pool represents an unexpected mechanism of homeostatic plasticity.

We also performed experiments in which ISCs were completely ablated, and observed no recovery of the ISC pool over the lifespan of flies. This is consistent with another recent report in which ISCs were completely ablated (Lu and Li, 2015). In both of these cases the *esg-Gal4* driver was used for depletion, allowing the conclusion that there are no esg^- ISC precursors in the adult fly. Interestingly, in contrast to a recent report that found that flies lacking ISCs had almost normal lifespans (Resende et al., 2017), we found that complete ISC ablation reduced fly

Figure 4. ISC Proliferation Rate Regulates Midgut Epithelial Cell Turnover

(A–C) Midgut epithelial cell turnover (GFP; green) was decreased in midguts overexpressing cell-cycle inhibitors (+Dap+Wee1) for 21 days with $esg^{ts}F/O$ compared with midguts expressing GFP alone for 21 days. Prospero$^+$ enteroendocrine cells are in red.

(D and E) The surface area and the total R4a + b cell number of midguts overexpressing Dap and Wee1 for 21 days (n = 16) with $esg^{ts}F/O$ was similar to midguts expressing GFP alone for 21 days (n = 10; n = 6 for 1 day).

(F) Percent midgut epithelial turnover (%GFP$^+$ cells per total R4a + b cell number) was reduced in midguts overexpressing Dap and Wee1 for 21 days with $esg^{ts}F/O$ compared with control midguts expressing GFP alone for 21 days. The data in (A) to (F) suggest that decelerating ISC proliferation increases midgut cell lifespan and decreases their turnover.

(G and H) Midgut epithelial turnover (GFP; green) was increased in midguts expressing secreted Spi (sSpi) for 7 days with $esg^{ts}F/O$ compared with control midguts expressing GFP alone for 7 days.

(I and J) While the surface area mildly increases in midguts expressing sSpi for 7 days (n = 7) with $esg^{ts}F/O$ compared with midguts expressing GFP alone for 7 days (n = 8), the total number of R4a + b cells was similar between midguts expressing either sSpi or GFP alone for 7 days.

(K) Percent midgut epithelial turnover (% GFP$^+$ cells per total R4a + b cell number) was, however, increased in midguts expressing sSpi for 7 days with $esg^{ts}F/O$ compared with control midguts expressing GFP alone for 7 days.

(L) Turnover of GFP$^-$ cells was increased in midguts expressing sSpi for 7 days with $esg^{ts}F/O$ compared with control expressing GFP alone. The data in (G) to (L) suggest that accelerating ISC proliferation increases midgut cell turnover.

Surface areas and cell numbers in region R4a + b were measured on one side of the midgut epithelium. Total R4a + b cell number, % GFP$^+$ cells/total R4a + b cell number, and GFP$^-$ R4a + b cell number were determined from n = 5 R4a + b regions. Statistical significance was determined by unpaired t test (*$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$; ns [not significant] $p > 0.05$). In all plots the median is shown, box includes 25th–75th percentiles, and whiskers show minimum to maximum. DNA in (A–C), (G), and (H) (blue). Scale bars in (A–C), (G), and (H) represent 40 μm. See also Figure S4.

survival. Our data suggest that while flies can survive partial ISC loss for at least 4 weeks, complete ISC depletion results in a loss of midgut homeostasis and reduced survival.

The dynamics of ISC pool maintenance in the fly midgut have significant differences from those in the mammalian intestine. Symmetric ISC lineages are very often observed in the mouse intestine, while only 10% of ISC lineages are typically symmetric in the fly midgut (Figure 3) (de Navascues et al., 2012; O'Brien et al., 2011). Strikingly, murine ISC pools readily recover after stem cell depletion whereas in the fly ISC depletion appears to be irreversible. Furthermore, in the murine intestine, selection of stem cells based on niche occupancy is important (Barker, 2014), and partially differentiated TA cells can revert into ISCs if they can access the niche. These phenomena are not observed in the fly midgut, which has a dispersed niche, no TA cells, and a fixed number of ISCs. The different behavior of ISCs in these two species could be due to differing requirements for stem cell capability. The mouse's lifespan is more than ten times longer than the fly's, so murine ISCs need to maintain gut homeostasis for much longer. Mammalian ISCs have to renew themselves many more times during the host's lifespan and also accumulate more genomic damage from DNA replication, which could alternatively drive cell death or transformation. Perhaps because of these pressures, the mammalian intestine evolved a more flexible system for stem cell pool control, which allows both better recovery from injury and the capability to select defective ISCs while maintaining a normal-sized stem cell pool.

EXPERIMENTAL PROCEDURES

Fly Genetics

For stem cell ablation analysis, transgene expression was induced using the *esg*^{ts} system at 29°C and turned off at 18°C. Flies were shifted back to 29°C to induce GFP expression for 12 hr before dissection. The number of GFP^{+} cells was quantified manually using a cell counter under microscope. For clonal analysis, the "twin-spot" analysis clones were induced by heat shocking 3- to 5-day-old flies at 34°C in a water bath for 20 min and the heat-shocked flies were kept at 25°C to reduce the stress caused by heat shock. Clone size was then measured 16 hr after clone induction. For examination of midgut epithelial turnover, ISC clones were induced using *esg*^{ts}*F/O* by shifting flies to 29°C for 1, 7, or 21 days.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2017.04.002.

AUTHOR CONTRIBUTIONS

Y.J. designed and performed experiments shown in Figures 1, 2, 3, S2, and S3, and wrote the paper. P.H.P. designed and performed experiments shown in Figures 4, S1, and S4 and wrote the paper. B.A.E. conceived the project and wrote the paper. A.K. contributed to early stages of the work. C.Z. and B.P. helped with experiments in Figures 1, 2, and 3.

ACKNOWLEDGMENTS

We acknowledge DFG SFB873 and ERC AdG 268515 to B.A.E., and the Helmholtz Gemeinschaft (DKFZ A220) for support.

Received: May 9, 2016
Revised: April 3, 2017
Accepted: April 4, 2017
Published: May 4, 2017

REFERENCES

Barker, N. (2014). Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat. Rev. Mol. Cell Biol. *15*, 19–33.

Buczacki, S.J., Zecchini, H.I., Nicholson, A.M., Russell, R., Vermeulen, L., Kemp, R., and Winton, D.J. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature *495*, 65–69.

de Navascues, J., Perdigoto, C.N., Bian, Y., Schneider, M.H., Bardin, A.J., Martinez-Arias, A., and Simons, B.D. (2012). Drosophila midgut homeostasis involves neutral competition between symmetrically dividing intestinal stem cells. EMBO J. *31*, 2473–2485.

Dutta, D., Dobson, A.J., Houtz, P.L., Glasser, C., Revah, J., Korzelius, J., Patel, P.H., Edgar, B.A., and Buchon, N. (2015). Regional cell-specific transcriptome mapping reveals regulatory complexity in the adult drosophila midgut. Cell Rep. *12*, 346–358.

Jiang, H., and Edgar, B.A. (2011). Intestinal stem cells in the adult *Drosophila* midgut. Exp. Cell Res. *317* (19), 2780–2788.

Jiang, H., Patel, P.H., Kohlmaier, A., Grenley, M.O., McEwen, D.G., and Edgar, B.A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. Cell *137*, 1343–1355.

Jiang, H., Grenley, M.O., Bravo, M.J., Blumhagen, R.Z., and Edgar, B.A. (2011). EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in *Drosophila*. Cell Stem Cell *8*, 84–95.

Lopez-Garcia, C., Klein, A.M., Simons, B.D., and Winton, D.J. (2010). Intestinal stem cell replacement follows a pattern of neutral drift. Science *330*, 822–825.

Loudhaief, R., Brun-Barale, A., Benguettat, O., Nawrot-Esposito, M.P., Pauron, D., Amichot, M., and Gallet, A. (2017). Apoptosis restores cellular density by eliminating a physiologically or genetically induced excess of enterocytes in the *Drosophila* midgut. Development *144*, 808–819.

Lu, Y., and Li, Z. (2015). No intestinal stem cell regeneration after complete progenitor ablation in *Drosophila* adult midgut. J. Genet. Genomics *42*, 83–86.

Micchelli, C.A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. Nature *439*, 475–479.

O'Brien, L.E., Soliman, S.S., Li, X., and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. Cell *147*, 603–614.

Ohlstein, B., and Spradling, A. (2006). The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. Nature *439*, 470–474.

Ohlstein, B., and Spradling, A. (2007). Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential notch signaling. Science *315*, 988–992.

Patel, P.H., Dutta, D., and Edgar, B.A. (2015). Niche appropriation by *Drosophila* intestinal stem cell tumours. Nat. Cell Biol. *17*, 1182–1192.

Resende, L.P., Truong, M.E., Gomez, A., and Jones, D.L. (2017). Intestinal stem cell ablation reveals differential requirements for survival in response to chemical challenge. Dev. Biol. *424*, 10–17.

Roth, S., Franken, P., Sacchetti, A., Kremer, A., Anderson, K., Sansom, O., and Fodde, R. (2012). Paneth cells in intestinal homeostasis and tissue injury. PLoS One *7*, e38965.

Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M., and Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature *469*, 415–418.

Snippert, H.J., van der Flier, L.G., Sato, T., van Es, J.H., van den Born, M., Kroon-Veenboer, C., Barker, N., Klein, A.M., van Rheenen, J., Simons, B.D., et al. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell *143*, 134–144.

Tian, H., Biehs, B., Warming, S., Leong, K.G., Rangell, L., Klein, O.D., and de Sauvage, F.J. (2011). A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature *478*, 255–259.

Wang, L., Zeng, X., Ryoo, H.D., and Jasper, H. (2014). Integration of UPRER and oxidative stress signaling in the control of intestinal stem cell proliferation. PLoS Genet. *10*, e1004568.

Xiang, J., Bandura, J., Zhang, P., Jin, Y., Reuter, H., and Edgar, B.A. (2017). EGFR-dependent TOR-independent endocycles support *Drosophila* gut epithelial regeneration. Nat. Commun. http://dx.doi.org/10.1038/ncomms15125.

Yan, K.S., Chia, L.A., Li, X., Ootani, A., Su, J., Lee, J.Y., Su, N., Luo, Y., Heilshorn, S.C., Amieva, M.R., et al. (2012). The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. Proc. Natl. Acad. Sci. USA *109*, 466–471.

Yu, H.H., Chen, C.H., Shi, L., Huang, Y., and Lee, T. (2009). Twin-spot MARCM to reveal the developmental origin and identity of neurons. Nat. Neurosci. *12*, 947–953.